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Authors
Ho, Dean
Zarrinpar, Ali
Chow, Edward Kai-Hua

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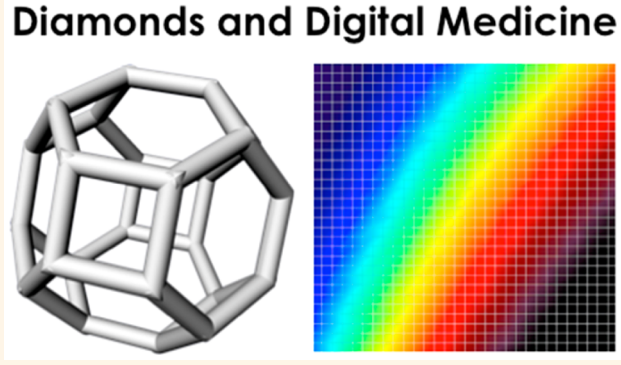
Diamonds, Digital Health, and Drug Development: Optimizing Combinatorial Nanomedicine

Dean Ho,[*,†,‡,§,∥,⊥] Ali Zarrinpar,[*,⊥,#,¶] and Edward Kai-Hua Chow[*,▲,▽]

[†]Division of Oral Biology and Medicine, School of Dentistry, [‡]The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, [§]Department of Bioengineering, Henry Samueli School of Engineering and Applied Science, [∥]California NanoSystems Institute, [⊥]Jonsson Comprehensive Cancer Center, [#]Department of Surgery, Division of Liver and Pancreas Transplantation, David Geffen School of Medicine, and [¶]Dumont-UCLA Liver Transplant and Cancer Center, University of California, Los Angeles, California 90095, United States

[▲]Cancer Science Institute of Singapore and [▽]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117599

ABSTRACT: The field of nanomedicine has already seen substantial progress in the clinic, with multiple formulations being evaluated through clinical studies. From poly(lactic-*co*-glycolic acid) and cyclodextrin-based drug-delivery platforms to metallic nanoparticles for photothermal treatment and imaging, nanotechnology has enabled versatile strategies to treat and to diagnose a wide range of disorders. However, as the field as a whole pushes forward, barriers that have always challenged conventional drug development have already started to impact nanomedicine translation. These include exorbitant costs, substantial time to development, and the uncertainty of achieving major improvements in efficacy or safety. Of note, there has been, until recent advances, a virtual inability to identify optimal drug doses either as monotherapies or components of combination therapy. In this Nano Focus, we assess how the impact of nanotechnology in the clinic can be optimized through systematically designed combinatorial nanotherapy. In addition, we provide a clinical perspective on how a recently unveiled technology platform can substantially alter the landscape of combinatorial nanomedicine, drug development, as well as conventional drug development.



Nanomedicine formulations have made important strides in clinical and preclinical studies toward a variety of indications.[1−5] As additional classes of nanomaterials progress toward clinical validation, an emerging area of interest that has arisen is the inclusion of these nanocarriers in multi-drug treatment regimens, which represent the standard of care for indications ranging from infectious disease to cancer. To explore the benefits of nanomedicine-enabled combinatorial delivery, several important studies have examined either delivering different nanomodified formulations in combination, or loading a combination of agents onto one nanoparticle with promising results.[6−9] However, in addition to carefully determining the active agents being delivered, which is one of the important attributes of combination therapy, the dose ratio of these agents has a profound impact on efficacy and safety. In fact, drug synergy and antagonism, among other important properties, can be altered even through minute changes in drug−dose ratios. The problem, however, is that up until now there has not been an effective way to determine the optimal drug−dose ratio of a nanoparticle carrying multiple agents (e.g., siRNA, small molecule therapies, biologics, *etc.*), or of a combination composed of multiple classes of nanoparticles, a combination comprised of one class of nanoparticle codelivered with unmodified drugs, or even a combination composed entirely of unmodified drugs (conventional combination therapy). This is understandable, since the true dosing parameter space is insurmountably large, especially with increasing numbers of drugs in the combination. In this Nano Focus, we examine the recent progress of nanomedicine translation toward the clinic, and subsequently address the larger issue of how the field of nanomedicine can maximize its impact in the clinic by systematically using its arsenal of therapies to optimize multi-drug treatment, circumventing the challenges of drug development that have confronted the community.[10−15]

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Beyond Monotherapy with Nanotechnology. As nanomedicine formulations continue to be assessed in the clinic, an array of emerging nanomaterials are seeing promising safety and efficacy in preclinical and recent clinical studies (NCT01612546, NCT01620190, NCT02178436, NCT02769962, NCT02106598).[16−22] As nanomedicine formulations are initially translated toward patient studies, the implementation of conventional drug development protocols to assess their efficacy and safety has often resulted in monotherapy trials. However, when these formulations are developed for indications that are best treated with combination therapy, it is becoming increasingly evident that the codelivery of multiple investigational drugs may be more effective compared to clinical standards.[23] This strategy could include the formulation of a combination therapy based on multiple nanomedicines or an investigational nanomedicine combined with multiple drugs (repositioned or approved), among other strategies. Certainly, conducting a trial using nanotechnology-enhanced monotherapy is an important component of the current regulatory pathway to understand single-agent efficacy and safety better. However, much like conventional drug development, monotherapy may be the first step toward unleashing the potential of nanomedicine so that the field can substantially affect clinical care through combinatorial delivery of multiple nanotechnology-enhanced agents.

Optimizing (Nano)Medicine from the Top Down. To address the challenge of developing improved combination therapies in both nanomedicine and broader drug development, many important approaches have been examined. The components include dosing algorithms, pharmacokinetic modeling, systems biology, genome-guided therapy, drug synergy-based prediction, and high-throughput screening, among others.[24−26] While these approaches have identified compounds that may serve as new strategies for treating certain disorders, they are not geared toward determining optimal drug−dose ratios and are thus not capable of determining maximally efficacious drug combinations. This situation is especially evident when a disease mechanism is used to guide drug selection. While this mechanistic approach can help suggest candidates, it cannot determine the dose, which has a profound impact on treatment outcomes. In fact, the dose ratio dependence of drug synergy and antagonism and the fact that these doses can vary substantially among *in vitro*, preclinical, and clinical administration explains why the use of synergistic drug combinations alone does not predict clinical trial success or maximal efficacy. Therefore, identifying both the optimized composition and dose ratios of drug combinations is required to maximize treatment outcomes, as these parameters are correlated to each other.

Recently, a technology platform named phenotypic personalized medicine (PPM) was developed to identify drug candidates and to optimize drug ratios simultaneously without the need for complex disease mechanism information. In addition, when presented with even a large pool of drugs within a parameter space that prohibitively requires billions of tests, PPM effectively maps out this entire space and pinpoints both the drugs and the drug ratios that result in the best possible treatment, backed by experimental data. In addition, PPM does not rely on algorithms, predictive modeling, or even disease biology. It is a top-down technology that is disease-mechanism-independent. While disease biology is not used to drive drug development using this approach, its ability to optimize treatment outcomes serves as a powerful starting point to uncover the important biological mechanisms at the foundation of these outcomes and possibly even to introduce additional drug candidates to target these mechanisms. At its foundation, PPM is a digital medicine platform that treats the biological system (cell, preclinical model, patient) as a black box and determines and correlates the inputs (drug candidate, dose, any form of therapy) with a quantifiable output. Examples of outputs include tumor burden, viral/bacterial load, international normalized ratio, target trough levels, *etc.* Phenotypic personalized medicine achieves this task by pinpointing population or patient-based constants that are calibrated through experimental and clinical therapy. These attributes make PPM indication agnostic and applicable to all diseases and drug classes ranging from oncology to cardiovascular medicine and from small molecule and biologics to immunotherapy.

With regard to combinatorial nanomedicine design, PPM is applicable toward all classes of nanoparticle and therapeutic agents. To demonstrate the impact that systematic optimization can have on combination therapy outcomes, PPM was used to pinpoint drug−dose ratios that resulted in the best possible safety and efficacy of a multi-drug treatment using nanodiamond (ND)-modified drugs. Nanodiamonds have recently emerged as promising drug delivery and imaging agents. They have primarily been explored as monotherapy-delivering or biomaterial-fortifying platforms. For example, a ND−doxorubicin complex (NDX) mediated marked improvement in the efficacy and safety of preclinical liver, breast, and brain cancer treatment using administration routes that included systemic (tail vein) and localized (convection-enhanced delivery) administration routes.[27−30] In addition, NDs have mediated among the highest ever reported per-gadolinium relaxivity values compared to those of all nanoparticle and clinical agents.[31] Recently, a clinical study to validate ND-modified biomaterials toward root canal therapy was initiated (NCT02698163).

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In a study on breast cancer, PPM was used to optimize drug ratios for a four-drug combination containing three ND-modified drugs (ND−doxorubicin, ND−bleomycin, ND−mitoxantrone) and unmodified paclitaxel. The disease was represented by three different breast cancer lines (MDA-MB-231, BT-20, MCF-7), and three control cell lines (H9C2, IMR90, MCF10A) were used in order to optimize the therapeutic window based on maximal cancer cell death and minimized control line death (Figure 1).[32] Interestingly, PPM optimization showed that arbitrarily constructed drug combinations often resulted in negative therapeutic windows based on more control cell death compared to cancer cell death. In fact, systematic PPM optimization was required to identify specific drug ratios that resulted in maximal cancer cell death and minimal control cell death. Of note, PPM-optimized ND−

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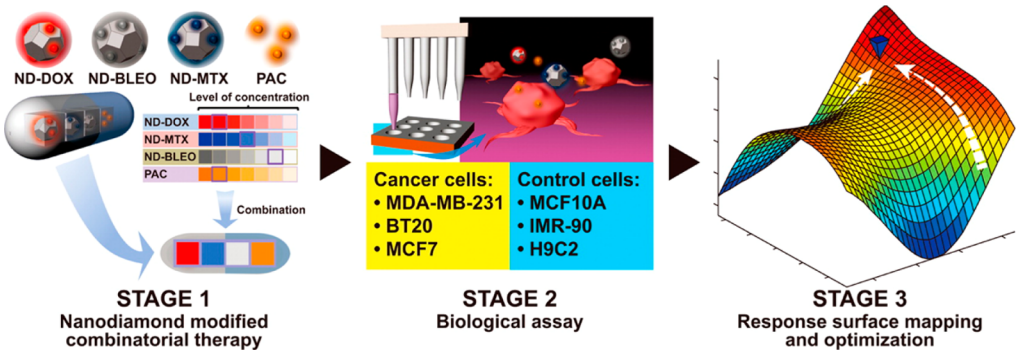

Figure 1. Optimization of combinatorial nanomedicine. Phenotypic personalized medicine was used to pinpoint the optimal ratios in a combination composed of nanodiamond–doxorubicin, nanodiamond–bleomycin, nanodiamond–mitoxantrone, and unmodified paclitaxel. This was achieved independent of disease mechanism and resulted in optimized, nanodiamond drug combinations that outperformed single-drug administration and randomly constructed drug combinations. Reproduced from ref 32. Copyright 2015 American Chemical Society.

drug combinations outperformed both ND-modified and unmodified single-drug therapy and randomized ND-modified and unmodified multi-drug treatment. Importantly, PPM-optimized ND–drug combinations also outperformed PPM-optimized unmodified drug combinations. This result is an indication that the nanomaterial itself should be considered an input that is subject to dose ratio determination. This observation makes systematic optimization of nanomedicine combinations even more important when translating these investigational therapies into patients.

In this particular study, the four-drug combination was predetermined as a proof-of-concept study to integrate PPM with nanomedicine optimization. One of the main advantages of PPM, however, is its ability to realize and to validate previously unexplored drug combinations through an unbiased optimization process. This is a critically important point since PPM has shown that biased drug combination design (*e.g.*, combining therapies based on presumed synergies) rarely results in an optimal treatment outcome.[33] When PPM was applied toward identifying optimal drug combinations against liver cancer through targeting glucose metabolism, previously unknown interactions between key signaling pathways were identified as possible therapeutic approaches. A pool of 80 kinase inhibitors were initially screened for both enhanced liver cancer cell killing compared to normal hepatocytes as well as glucose uptake inhibition. Five lead kinase inhibitors targeting Src, Janus kinase 3 (JAK-3), fetal liver kinase-1 (Flk-1), platelet-derived growth-factor-receptor kinase (PDGFRK), and cyclic AMP-dependent protein kinase (PKA)/cyclic GMP-dependent protein kinase (PKG) were chosen for PPM evaluation using the hepatic cancer cell line, Hep3B, compared to normal hepatocytes (THLE-2). The PPM analysis identified several unidentified drug combinations, including the use of JAK-3 inhibitors with PKA/PKG inhibitors. This study identified a potential role for JAK-3 in glucose metabolism in liver cancer as well as a critical interaction between JAK-3 and PKA/PKG in liver cancer cell survival. Thus, PPM's unbiased approach can be used to identify optimal drug combinations that may be mediated by molecular mechanisms that have yet to be discovered and would normally not be tested using conventional rational approach methods to drug combination design.[34]

As a digital medicine platform that is capable of pinpointing the constants that characterize a biological system's phenotypic response to therapy, PPM makes dose ratio identification possible as well as efficient. Of note, the drug–dose ratios identified through *in vitro* testing are used solely to eliminate ineffective drug candidates. To eliminate the use of treatment response to therapy predictions to guide combination design, PPM takes the lead combinations identified at this stage and reoptimizes them at the preclinical and clinical stages to maximize efficacy and safety by implicit validation.

As nanomedicine continues to translate to the bedside, clinical optimization of combination therapies will become increasingly important.

Optimization and Personalization for Clinical (Nano)-Medicine. Combination therapy is the standard of practice in the clinical care of diseases as diverse as leprosy and diabetes. Furthermore, as people increasingly suffer from comorbidities, multiple treatments also confound their care. Even when there are single-drug treatments available for a condition, inter-individual variability with regard to genetics, environment, and physiology is usually not adequately taken into account by the standard population-based treatment approaches that have led to the recommended dosing protocols. Individualized drug dosing is often difficult even with single-drug regimens. When multiple drugs are required, the task is next to impossible and requires several titration steps and trial and error to reach the desired effect. As nanomedicine continues to translate to the bedside, clinical optimization of combination therapies will become increasingly important.

Demonstrating the importance of pinpointing doses and their impact on multi-drug delivery, a recent pilot clinical trial individualized post-transplant immunosuppression in patients.[35] In solid organ transplant immunosuppression, patients are assigned a target range for the blood trough level of tacrolimus. This represents the current standard for assessing the efficacy of immunosuppression. When tacrolimus levels are too high, patients can experience neurotoxicity and nephrotoxicity. When trough levels are too low, organ rejection may occur. The key finding from this clinical trial demonstrated that a parabola represents the phenotypic response of all patients to drug administration. In this study, drug doses (input) and trough levels (output) were used to calibrate the patient phenotypic response and to obtain the individualized constants from which their personalized parabolic surfaces were constructed (Figure 2). These parabolic maps implicitly account for patient/disease heterogeneity, genetics, disease biology/mechanism, and pharmacokinetics, among other

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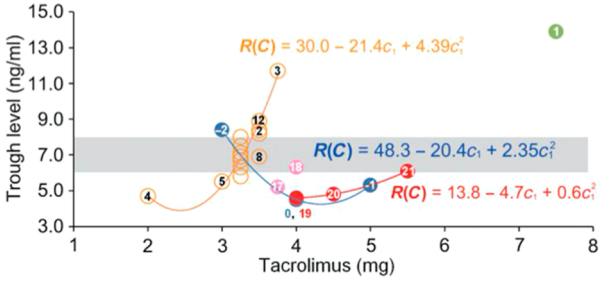

Figure 2. Individualization of clinical immunosuppression. Phenotypic personalized medicine was capable of calibrating patient response to therapy to construct parabolic maps to pinpoint patient-specific dosing parameters. This figure represents the parabolic calibration process for a single patient during the course of post-transplant immunosuppression. The immunosuppressant tacrolimus (*x*-axis) dose was used as the input, while tacrolimus trough level in the blood (*y*-axis) was used as the output. The patient-specific target trough level (shaded region) was physician-determined. Each parabola and corresponding constants represent the patient-specific clinical response to therapy while under a specific drug regimen. The numbers in the respective data points represent the treatment days. As patients experience regimen changes during their care, a recalibration process results in a new parabola and new constants, represented by the changes in color in the figure. Using this dynamically actionable technology platform, continuous optimization was performed to individualize therapy for the entire duration of care. Reproduced with permission from ref 35. Copyright 2016 American Association for the Advancement of Science.

factors and do not require this explicit information to tailor treatment continuously. These maps were then used to pinpoint the drug dose needed to reach the target trough level. Of note, each patient's regimen was highly variable. For example, frequent changes to the doses of antifungals, anti-inflammatories, antiviral medications, and other agents; the addition or discontinuation of therapies; as well as procedures such as hemodialysis resulted in changes to the patient constants that were recalibrated to construct new parabolas. This process resulted in the continual optimization of drug treatment for the entire course of therapy for each patient involved in the study. Disease biology and genome-guided therapy, as well as pharmacokinetics-based modeling and pharmacogenomics, among other mechanism-driven approaches are not geared toward this level of dynamic and actionable optimization. This degree of individualization of care confirmed the importance of identifying the right drug doses during multi-drug treatment because drug synergism and antagonism were shown to be both dose-dependent and patient-dependent. This was demonstrated in a patient-specific shift in cotrimoxazole and tacrolimus interaction during the course of multi-drug treatment (Figure 3). With regard to trial end points, PPM-managed patients had 50% fewer substantial deviations (defined as greater than 2 ng/mL) from the target trough levels compared to control patients. In addition, PPM-managed patients were discharged nearly 3 weeks earlier than control patients. While time to discharge is not a standard metric for immunosuppression outcomes, it does serve as an indicator for the potential broader implementation of the PPM approach beyond dosing technology; PPM can also serve as a valuable digital health platform to optimize multi-drug treatment as well as clinical trial drug administration protocols.

CONCLUSIONS AND PROSPECTS

As this clinical study and preclinical studies demonstrate, the field of nanomedicine and the broader drug development community as a whole no longer need to default solely to

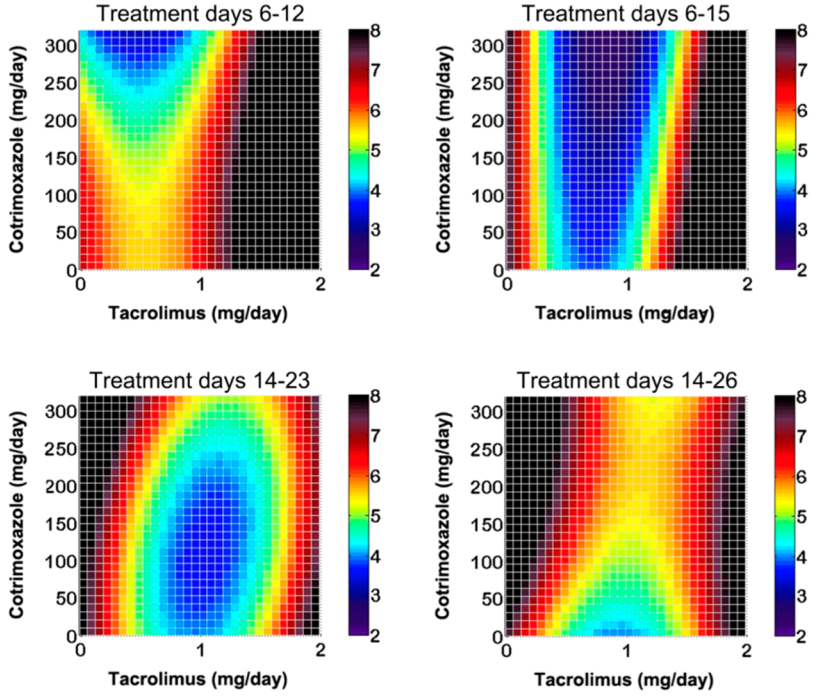

Figure 3. Dose-dependent drug synergism and antagonism. Clinical personalization of drug administration revealed that drug synergism and antagonism shift over time and are drug–dose-dependent. This patient's drug response map revealed a shift in the interaction between cotrimoxazole and tacrolimus during the course of treatment. Reproduced with permission from ref 35. Copyright 2016 American Association for the Advancement of Science.

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approaches like drug screening to identify potential new therapies, especially when there is more to the story than lead candidates alone. When the entire dosing space can be explored for optimal combinations and drug−dose ratios, the community can likely move beyond conventional combination therapy design to develop new treatments and move beyond conventional dose escalation to test new medicines. Furthermore, relinquishing the ability to optimize drug−dose ratios no longer needs to be the standard of drug development and testing. The community now has a chance to realize all of the benefits of nanotechnology in the clinic if an arsenal of important technologies like PPM bolsters combinatorial nanomedicine development.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: dean.ho@ucla.edu.
*E-mail: azarrinpar@mednet.ucla.edu.
*E-mail: csikce@nus.edu.sg.

Notes

The authors declare the following competing financial interest(s): D.H., A.Z., and E.C. are co-inventors on nanodiamond and personalized medicine patents.

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